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THE RELATION BETWEEN THE DENSITY OF CELL SAPS AND THE FREEZING POINTS OF LEAVES.[1]

WILLIAM WOODWARD OHLWEILER.

INTRODUCTION.

The optimum temperatures for plant growth vary greatly for different regions, and the ranges of variation for different plants are almost as varied as the plants themselves. Where plants are grown under glass it is necessary to vary the temperature according to the requirements of the plants to be grown, as is shown by the use of tropical, subtropical, stove, temperate, and cool houses. Some plants are closely limited to these temperatures, while others seem to be more or less cosmopolitan. However, for the species and varieties of plants native to any one region, there is this same variation in the temperature requirements, some seeking the cool, wet bogs, and others the dry, sunny slopes. We have, in any region, plants that are very susceptible to cold periods, and others that are not. Late spring frosts destroy some crops with apparent regularity, while others remain unharmed. Why is there this difference? It could hardly be hereditary, since long years of acclimatization would have eliminated the weaklings. The structure of the cell walls might determine whether or not cells of certain plants would freeze. However, it is not the freezing of the cell wall which kills plants, but the freezing of the cell contents. It is more or less true that the cell walls may have some effect in retarding or preventing the freezing of the contents, but this, when we consider the small amount of tissue involved, would have but little influence upon the

[1] A thesis presented to the Faculty of Washington University in candidacy for the degree of Master of Arts, June, 1911.

final result. Can we then narrow our argument down to the ability of the cell sap to resist freezing?

In the latter part of April, 1910, there occurred throughout the State of Missouri, a drop in temperature to 25°F. Foliar growth of the trees and shrubs was in an advanced condition, and the freeze, which was accompanied by rain and snow, did a great deal of damage to all green material, but, as is the case generally, destroyed the foliage of some trees and shrubs more than of others. As a matter of personal interest, the damage to foliage was recorded in detail, and these notes occur in this paper as Table 1. From these observations alone, no conclusions as to the reason for the difference in resistance to freezing were apparent. Many plants which would reasonably have been expected to behave the same, acted quite differently, and the problem became more perplexing the more it was studied.

A year later, *i. e.,* in the spring of 1911, it was decided to extract the saps of the various leaves, freeze them artificially, and determine their freezing points, thereby ascertaining if any correlation existed between this artificial behavior and that actually exhibited in the freeze of the year before. These results and conclusions are the basis of this paper. Only trees and shrubs growing in the Missouri Botanical Garden, out of doors, were observed, and their native habitats, *fide* Index Kewensis, are given in Table 1. As a matter of convenience it has seemed advisable to separate the results of natural from artificial freezing, the two being essentially different investigations, and to summarize in the conclusion such correlations as may occur.

The paper then comprises:—

1. Notes on the effect of the freeze of April 24, 1910, on the trees and shrubs growing in the Missouri Botanical Garden, their recovery and other data.

2. The freezing point of leaf saps, determined artificially, and notes in connection therewith.

3. Conclusions.

1. NOTES ON THE EFFECT OF THE FREEZE OF APRIL 24, 1910, ON THE TREES AND SHRUBS GROWING IN THE MISSOURI BOTANICAL GARDEN, THEIR RECOVERY AND OTHER DATA.

The continental position of Saint Louis is such, that it is practically unaffected by the proximity of large bodies of water or mountain ranges. It lies in a large river valley about midway between the divides of the Appalachian and Rocky Mountain ranges. In every direction the country is open for hundreds of miles, except as it is interrupted by the retaining walls of the two mountain ranges already referred to. To the south lies the Gulf of Mexico, and it is, as might be expected, one of the storm-forming areas of this section. The storms from this section are in the main warm, and although often destructive, need not be considered in connection with this paper. To the north the storm area is more varied, and the storms that sweep down from this section are usually very cold, and often very sudden. Since east and west need not be taken into consideration as affecting temperature changes directly, they may indirectly be responsible for subjecting Saint Louis to the extremes of temperature from the north and south, *i. e.,* they form the channel through which these winds are forced to blow. Since it is unaffected by the tempering influence of large bodies of water, its frost period is much later than that of cities of corresponding latitude on the oceans, or near them. (Dorschied, Metr. Zeitschr. **24**: 11-24. *f. 1.;* 49-64. *pl. 1.* 1907.) Dorschied places Saint Louis on the same line, as regards beginning, ending and mean duration of frosts, as points on the coast of Alaska, British Columbia, New England, Southern Iceland, Norway, and the Western Alpine and Mediterranean regions. The average date of the last killing frost for Saint Louis is April 2. (Garriott, E. B. Notes on Frost. Farmers' Bull. U. S. Dept. Agr. 104: 16. 1910.) The prevailing direction of the wind during the month of March, 1910, was from the southwest, and means that this was a warmer month than usual. Quoting the summaries for March and

April (U. S. Dept. Agr., Weather Bur., Monthly Metr. Summary for Saint Louis, Mo., March and April, 1910):—

"The weather for March was very unusual. The mean temperature was 57.5°, which is 3.2° higher than for any previous March, and the temperature was continuously above the normal, except on the 9th, 10th, 14th, and 15th. The maximum temperature for the month was 87, and this has been exceeded but once in March in the history of the station. Freezing temperatures occurred on three days only. The precipitation was the lowest ever recorded for March, and only twice in forty years has a smaller amount been recorded for any month. The number of clear days, 22, is the highest ever observed in March, and the number of cloudy days, 2, the lowest. The sunshine was 79 per cent of the possible amount, and was the greatest for March since the records began. During the latter part of the month the atmosphere was very hazy, causing the sun and the moon to appear very red. The wind movement, while not the least for any March, was much below the average, and a less amount has been recorded five times only in March."

and for April:—

"The temperature for April averaged slightly below the normal and was characterized by great extremes, the maximum being surpassed but once in the history of the station. The minimum, while not the least for any April, was unusual for the last decade of the month. All previous low temperatures have been recorded in the first half of the month. The minimum of 25° on the 24th was the lowest minimum after the 20th in 74 years. The low temperature was caused by a slow movement of a storm area from Illinois to lower Michigan that developed in intensity and brought down cold air from the northwest. The mean temperature for April was 1.7° lower than that of March. A similar case occurred in April, 1907, when the mean temperature for April was 5.9° lower than March. The snowfall was the heaviest for any April, but never before has so great an amount fallen as late as the 24th of the month."

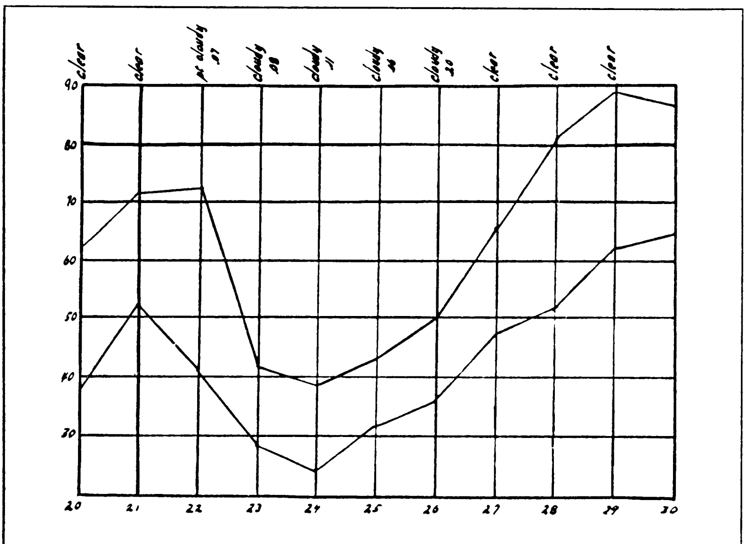
The location of the Garden on a high point of land, with well-drained areas sloping toward the west and northwest, makes damage from the average frost not a very common occurrence. Ground frost and consequent damage to tender herbaceous plants sometimes occurs, but often when frost has been known to occur on the ground, there has not been any noticeable effect on the leaves or flowers of trees and shrubs. The Garden is also slightly favored in

an ordinary frost by the fact that the major part of the slope is north and northwest. However, "the cold spell of April 24, 1910, was accompanied by cloudy skies and rain, mixed with snow, and the movement of the cold air currents was quite different from that which takes place under a clear sky, or when there is no rain or snow. It will be observed that during cold spells, with more or less cloudiness or moisture, the temperature on the hills may fall to a lower value than the temperature in the valleys. On the other hand, had clear weather prevailed * * * the temperature conditions would have been reversed, the valleys probably being colder." (Garriott, E. B. Notes on Frost. Farmers' Bull. U. S. Dept. Agr. 104: 12. 1910.) Moreover, the minimum temperature of the 24th was, as reported by the Weather Bureau, 25°F., and acted for a time long enough to insure freezing of such things as were in condition to freeze. As a matter of fact no frost was formed, and the results are simply those of direct freezing. This makes the later comparisons of natural and artificial freezing much more convincing than would be the case had the damage to vegetation been due to frost, for we could not be quite sure that we had duplicated frost conditions of temperature and moisture, while duplicating freezing temperatures is a comparatively easy matter.

The effect of cold upon vegetation in general depends very largely upon the rapidity with which the destructive change in temperature is brought about, being greater when the change takes place within narrow limits of time. Temperature changes in March cannot be said to directly concern the plants affected by a freeze during the next month, yet the same temperatures occurring a few days before a heavy freeze, could have serious results. In the one case acclimatization results, whereas in the other no adaptive change takes place. In how far the process of acclimatization can protect plants from extremes of cold we do not know, although the principle is applied in using cold frames to acclimatize former greenhouse plants. How-

ever, the case under consideration represents the reverse process, since the weather to which these plants were being subjected was gradually adapting them to warmer instead of colder weather. It would seem then, on the face of things, that frosts occurring in the spring find the plants, as a rule, preparing for the growing season and warmer weather, while those occurring in the fall and at the close of the growing season, find the plants tending to acclimatize themselves to the approaching cold weather. There should be no doubt, then, as to the period of the year in which a frost is the more destructive.

The immediate condition of the weather around the 24th of April, *i. e.*, the period from the 20th to the 29th deserves some attention, and the following temperatures, maximum and minimum, are here given, and are well illustrated by the appended diagram:—



in which the ordinates are the temperatures recorded for the ten days represented by the abscissae. The weather for

each day is recorded at the upper edge of the diagram, and the precipitation, if any, underneath the word "cloudy," "partly cloudy," etc., the precipitation for clear days being zero. The upper line is for the maximum, and the lower for the minimum temperature. It will be seen from the diagram that during a period of clear weather the temperature gradually rose until reaching the 72° mark, dropping from here to 28° the next night, and to the minimum of 25° the following night—the greatest drop for any 24-hour period being 44°. It is quite probable that the drop just referred to, was the one that did the greater part of the damage, although it is quite certain that the next two days of freezing, with the minimum at 25°, could leave but little to be desired for a good freezing test. Furthermore, the extent of the freeze was quite general over the entire state, and from no section was complete immunity recorded.

Foliar conditions in the main paralleled those of the weather. During the month of March conditions, except those of moisture, were very favorable for foliage growth. In spite of this, foliar growth was far in advance of the season. It would be difficult to show specifically the progress that vegetation had made, for it would be easier to tell what had not as yet started, but even this would not be possible, because all trees had leafed out somewhat. The stage of leafing is, then, merely one of degree. *Syringa vulgaris* had flowered, while at the same time *Catalpa* was still in the cluster or bunched condition, while *Sambucus canadensis* must have barely started. However, for determining the identical stages of growth in subsequent years the leaves of the Norway maple and the stage of growth of the *Syringa* may be taken as standards. Both of these were in full leaf.

The foliage of the trees immediately after the freeze presented an appearance that suggested fire. The northwest side of the trees was badly frozen in all cases, even on the evidently hardy material. Blackened, shapeless leaves, which

TABLE I.

Name of Plant	Habitat	Condition at the Time of Frost			Condition Several Days Later			Adventive Buds	Growth Two Weeks Later
		In Leaf	In Flower	In Bud	Effect on Leaf	Effect on new Shoot	Effect on Bud		
1. Vitis vinifera	Or., N. W. Ind.	Y	Y	?	K	K	K	G	N
2. Morus alba	Temp. As.	Y	Y	Y	K	PK	PK	G	N
3. Magnolia acuminata	N. Amer.	Y	Y	N	K	N	N	NG	Fair
4. Magnolia Yulan	China	Y	Past	N	K	N	N	N	Started
5. Liriodendron tulipifera	N. Amer. & China	Y	?	Y	K	PK	PK	G	Fair
6. Catalpa bignonioides	N. Amer.	Y	N	P	K	K	K	G	Slow
7. Fagus sylvatica purpurea	Europe	P	N	P	PK	N	N	N	Good
8. Aesculus Hippocastanum	As. Min.	Y	Y	N	PK	N	PK	N	Good
9. Ostrya virginica	N. Amer.	Y	N	N	PK	N	N	N	Good
10. Ginkgo biloba	Japan	Y	N	N	K	N	N	N	N
11. Fraxinus Ornus	Med. Reg., Or.	Y	N	N	K	PK	N	G	Fair
12. Rhus Toxicodendron	N. Amer.	Y	N	N	K	K	N	G	None
13. Koelreuteria paniculata	China	Y	N	Y	K	N	N	G	N
14. Cercis canadensis	N. Amer.	Y	Past	N	K	N	N	G	N
15. Cladrastis tinctoria	N. Amer.	Y	N	N	K	N	N	G	N
16. Hydrangea quercifolia	N. Amer.	Y	N	N	K	N	N	G	G
17. Fraxinus americana	N. Amer.	Y	N	Y	K	PK	N	G	N
18. Acer dasycarpum	N. Amer.	Y	Past	N	PK-N	N	N	N	G
19. Acer platynoides	Eu., Or.	Y	Past	N	PK-N	N	N	N	G
20. Gleditschia triacanthos	N. Amer.	Y	N	Y	K	PK	N	G	Slow
21. Paulownia imperialis	Japan	Y	N	Y	K	K	K	G	N
22. Clethra alnifolia	N. Amer.	N	N	N	N	N	N	N	Started
23. Ulmus campestris	Eu., Or.	Y	N	N	PK	N	N	N	G
24. Magnolia obovata discolor	Japan	Y	Y	Y	PK	N	N	N	Fair
25. Magnolia glauca	N. Amer.	P	N	Y	PK	N	N	N	Fair
26. Viburnum Opulus sterilis	Eu., N. As., Amer.	Y	Y	N	N	N	N	N	Good
27. Cornus Mas	Eu., As. Min.	Y	N	N	N	N	N	N	Good

TABLE I—Continued

Name of Plant	Habitat	Condition at the Time of Frost			Condition Several Days Later			Adventive Buds	Growth Two Weeks Later
		In Leaf	In Flower	In Bud	Effect on Leaf	Effect on new Shoot	Effect on Bud		
28. Rhus Cotinus atropurpureus	Med. Reg., Him. Reg., Or., China	Y	N	N	K	K	N	G	Slow
29. Chionanthus virginica	N. Amer.	Y	Y	N	K	K	N	G	Slow
30. Neillia opulifolia	N. Amer.	Y	N	N	PK	N	N	N	Good
31. Magnolia cordata	N. Amer.	P	N	Y	PK	N	N	N	Slow
32. Pinus Laricio (P. Austriaca)	S. Eu.	Old	N	Y	N	N	PK	N	Good
33. Pyrus arbutifolia erythrocarpa	N. Amer.	Y	Y	N	PK	N	N	N	Good
34. Aralia spinosa	N. Amer.	Y	N	Y	K	PK	N	G	Start
35. Platanus occidentalis	N. Amer.	Y	N	YP	PK	N	N	N	Slow
36. Quercus imbricaria	N. Amer.	Y	N	N	N	N	N	N	Good
37. Hydrangea paniculata	Japan	Y	N	N	PK	N	N	N	Good
38. Rhus typhina	N. Amer.	Y	N	Y	K	PK	N	G	Slow
39. Securingea fluggeoides	Japan	Y	N	N	K	PK	N	N	N
40. Taxodium distichum	N. Amer.	Y	N	N	K	?	N	N	N
41. Viburnum Lentago	N. Amer.	Y	Y	N	PK	N	Fls. PK	N	Good
42. Rhus copallina	N. Amer.	N	N	Y	N	N	N	N	N
43. Euonymus Sieboldiana	Japan	Y	Y	N	N	N	N	N	Good
44. Berberis vulgaris Thunbergii	Japan	Y	N	N	PK	PK	N	N	Fair
45. Rhamnus tinctoria	E. Eu., As. Min.	Y	N	N	PK	PK	N	N	Good
46. Spiraea van Houttei	Gardens	Y	Y	N	PK	Rarely	PK	N	Fair
47. Vitis hederacea (Ampelopsis quinquefolia)	N. Amer.	Y	N	Y	K	PK	N	N	N
48. Ilex quercifolia (I. opaca)	N. Amer.	Y	N	N	K	K	N	N	N
49. Magnolia Soulangeana	Gardens	Y	Past	Y	PK	N	N	N	Started
50. Lindera Benzoin	N. Amer.	Y	Y	N	PK	PK	N	N	Started
51. Magnolia Kobus	Japan	Y	N	N	N	N	N	N	Fine

TABLE II

Name of Plant	Habitat	Condition at the Time of Frost			Condition Several Days Later			Adventive Buds	Growth Two Weeks Later
		In Leaf	In Flower	In Bud	Effect on Leaf	Effect on new Shoot	Effect on Bud		
52. Ribes aureum	W. N. Amer.	Y	N	N	PK	N	N	N	Fine
53. Hamamelis virginiana	N. Amer.	Y	N	Y	PK	N	N	N	Slow
54. Philadelphus coronarius	S. Eu.	Y	N	N	PK	N	N	N	Fair
55. Philadelphus grandiflorus	N. Amer.	Y	N	N	PK	N	N	N	Fair
56. Halesia tetraptera	N. Amer.	Y	Past	N	PK	N	N	N	Fair
57. Diospyros virginiana	N. Amer.	Y	Past	N	K	K	N	G	N
58. Sassafras officinale	N. Amer.	Y	Past	N	K	K	N	G	N
59. Ailanthus glandulosa	China	Y	N	N	K	PK	N	G	N
60. Quercus tomentosa	Mexico	Y	N	N	PK	PK	N	Fair	Start
61. Fraxinus viridis	N. Amer.	Y	N	N	K	PK	N	G	N
62. Pyrus japonica	Japan	Y	Past	N	PK	N	N	N	G
63. Spiraea prunifolia	Japan	Y	N	N	PK	PK	N	N	G
64. Tsuga canadensis	N. Amer.	Y	N	N	K	K	N	N	G
65. Magnolia stellata	Japan	Y	Past	N	N	N	N	N	G
66. Taxus baccata	N. Temp. Reg.	Y	N	Y	K	K	N	G	Start
67. Diervilla rosea	China	Y	Y	N	N	N	N	N	Fine
68. Rosa rugosa	Japan	Y	N	Y	K	K	N	NG	Fair
69. Acanthopanax ricinifolium	Japan	Y	N	Y	K	N	N	N	Start
70. Xanthoceras sorbifolia	China	Y	N	N	K	N	N	N	Fair
71. Ligustrum amurense	Amur. Reg.	Y	N	N	PK	N	N	N	Good
72. Aesculus parviflora	N. Amer.	Y	N	N	PK	N	N	N	Good
73. Sophora japonica	Japan, China	Y	N	N	K	PK	N	G	N
74. Syringa persica	Cauc. Reg., Persia	Y	Past	N	N	N	N	N	Fine
75. Syringa vulgaris	Transylv.	Y	Past	N	N	N	N	N	Fine
76. Magnolia obovata purpurea	Gardens	Y	Past	N	PK	N	N	N	Slow
77. Cornus stolonifera	N. Amer.	Y	N	N	N	N	N	N	G
78. Hibiscus syriacus	Old World Calid.	Y	N	N	K	K	N	G	N

TABLE II—Continued

Name of Plant	Habitat	Condition at the Time of Frost			Condition Several Days Later			Adventive Buds	Growth Two Weeks Later
		In Leaf	In Flower	In Bud	Effect on Leaf	Effect on new Shoot	Effect on Bud		
79. Broussonetia papyrifera	Malay, Pacif. Ins.	Y	N	N	K	K	N	G	N
80. Acer laetum rubrum	Orient	Y	?	N	PK	N	N	G	Good
81. Acer saccharinum laciniatum	N. Amer.	Y	?	N	PK	N	N	G	Good
82. Amorpha fruticosa	N. Amer.	Y	N	Y	K	PK	N	N	G
83. Asimina triloba	N. Amer.	Y	P	N	K	K	N	G	Fair
84. Berberis ilicifolia	Magel. Reg.	Y	N	N	PK	PK	N	G	Fair
85. Calycanthus floridus	N. Amer.	Y	N	N	K	N	N	G	Fair
86. Carpinus Betulus	Eu., Cauc. Reg., Persia	Y	N	N	PK	PK	N	G	Fair
87. Cornus Bailey	Gardens	Y	N	N	PK	N	N	G	Good
88. Euonymus atropurpureus	N. Amer.	Y	N	N	PK	N	N	G	Good
89. Gleditschia aquatica	N. Amer.	Y	N	Y	K	PK	N	N	G
90. Gymnocladus canadensis	N. Amer.	Y	N	N	K	PK	N	G	Fair
91. Hydrangea radiata	N. Amer.	Y	N	N	PK	N	N	G	Fair
92. Juglans rupestris	W. N. Amer.	Y	N	N	K	PK	N	G	Fair
93. Leitneria floridana	N. Amer.	Y	N	N	K	N	N	N	Fair
94. Liquidambar styraciflua	N. Amer.	Y	N	N	PK	PK	N	G	Fair
95. Maclura aurantiaca	N. Amer.	Y	N	N	K	N	N	G	Good
96. Magnolia grandiflora	N. Amer.	Y	N	N	K	K	N	G	Fair
97. Magnolia Lennei ×	Gardens	Y	Past	N	PK	N	N	G	Poor
98. Magnolia Umbrella	N. Amer.	Y	N	N	PK	N	N	G	Best
99. Rhus glabra	N. Amer.	Y	N	N	K	PK	N	G	Fair
100. Robinia sp.	Hab?	Y	N	N	K	PK	N	G	Fair
101. Sambucus canadensis	N. Amer.	Y	N	N	K	N	N	G	Good
102. Tilia americana	N. Amer.	Y	N	N	PK	N	N	G	Good
103. Ulmus alata	N. Amer.	Y	N	N	PK	N	N	G	Good

later dried and fell to the ground, hung everywhere from the branches. Much of this dried material hung on for weeks, and in maples nearly all summer. This phenomenon of holding on to the dead leaves, or parts of leaves, was extremely interesting. The killing effect of the freeze extended in some cases, as in *Paulownia imperialis,* into the previous year's wood, and nearly always into the new shoots and foliar growth. The variation in its action is very well shown in Table I and II, in which the headings at the top of the columns are taken as questions, and answered for each of the species observed, by $Y =$ yes; $N =$ no or none; $K =$ killed; $PK =$ partly killed; $G =$ general or good.

From this table we are able to formulate a few general opinions, but mainly the fact that a freeze in its action on vegetation does not always have the same effect on all plants. When we admit this fact then, we are obviously led to the conclusion that there may be all gradations of cause and effect, but that when the cause is the same, as is the case in any definite freeze, then the effect is different for each particular case. In the case of the freeze under consideration it has been possible to group the effects under five rather clearly defined sections.

I. Those species for which complete immunity was recorded.

Clethra alnifolia	Magnolia stellata
Cornus Mas	Quercus imbricaria
Cornus stolonifera	Rhus copallina
Euonymus Sieboldiana	Syringa persica
Magnolia Kobus	Syringa vulgaris

II. Those in which the leaves were only partly frozen.

Acer dasycarpum	Euonymus atropurpureus
Acer laetum rubrum	Fagus sylvatica purpurea
Acer platanoides	Halesia tetraptera
Acer saccharinum laciniatum	Hamamelis virginiana
Aesculus Hippocastanum	Hydrangea paniculata
Aesculus parviflora	Hydrangea radiata
Cornus Baileyi	Ligustrum amurense
Diervilla rosea	Magnolia cordata

Magnolia glauca
Magnolia Lennei ×
Magnolia obovata discolor
Magnolia obovata purpurea
Magnolia Soulangeana
Magnolia Umbrella
Neillia opulifolia
Ostrya virginica
Philadelphus coronarius
Philadelphus grandiflorus
Platanus occidentalis
Pyrus arbutifolia erythrocarpus
Pyrus japonica
Ribes aureum
Taxodium distichum
Tilia americana
Ulmus alata
Ulmus campestris
Viburnum Lentago
Viburnum Opulus sterilis

III. Cases in which the leaf growth was completely killed, and in which there was no apparent injury to the new shoots.

Acanthopanax ricinifolium
Calycanthus floridus
Cercis canadensis
Cladrastis tinctoria
Ginkgo biloba
Hydrangea quercifolia
Koelreuteria paniculata
Leitneria floridana
Liriodendron tulipifera
Maclura aurantiaca
Magnolia acuminata
Magnolia Yulan
Sambucus canadensis
Xanthoceras sorbifolia

IV. Where new leaves were entirely killed, and the new shoots partially killed.

Ailanthus glandulosus
Amorpha fruticosa
Ampelopsis quinquefolia
Aralia spinosa
Berberis ilicifolia
Berberis vulgaris Thunbergii
Carpinus Betulus
Fraxinus americana
Fraxinus Ornus
Fraxinus viridis
Gleditschia aquatica
Gleditschia triacanthos
Gymnocladus canadensis
Juglans rupestris
Lindera Benzoin
Liquidambar styraciflua
Morus alba
Quercus tomentosus
Rhamnus tinctorius
Rhus glabra
Rhus typhina
Robinia sp.
Securinega fluggeoides
Sophora japonica
Spiraea prunifolia
Spiraea Van Houttei

V. New growth of all leaves and shoots completely killed.

Asimina triloba
Broussonetia papyrifera
Catalpa bignonioides
Chionanthus virginicus
Diospyros virginiana
Hibiscus syriacus
Ilex opaca
Magnolia grandiflora
Paulownia imperialis
Pinus austriaca
Rhus cotinus atropurpureus
Rhus toxicodendron

Rosa rugosa
Sassafras officinalis
Taxus baccata
Tsuga canadensis
Vitis vinifera.

SECTION I.

To the first may be added another as a result of this year's (1911) observations, namely *Lonicera involucrata,* which because of its behavior toward a frost of the present year is placed in the same category. The first in the list was the only plant that had not started and so was unaffected and was placed here. While the leaves of the plants recorded in this section were apparently frozen, if we may judge from outward appearances, still they did not show any after effects as were observed to appear in the second section. So that on this evidence alone they have been placed in the list of immune varieties, although it is not taken that they were not frozen. No notes of any peculiarities as to lateness of flowering, or early falling of leaves, etc., were taken later in the season, as will be evidenced in the resultant descriptions of the later sections.

SECTION II.

The enumeration of the species in the second section includes all those plants whose leaves, several weeks after the frost, showed, by the appearance of dried and withered parts, that sections had been killed as a direct or indirect result of the freeze. In nearly all cases the parts of the leaves took on a dried-up, rusty appearance, but in the case of *Cornus Baileyi,* the parts affected were red or black. In *Acer saccharinum laciniatum* the frozen parts dried up and in a few days were broken off by the wind, and only by a close observation could the effect of the freeze be ascertained, but in *Acer platanoides* and *Acer dasycarpum* the dried portions persisted, and during the summer it was possible to distinguish easily the frozen and unfrozen portions of the leaves. By this means it was easily seen from the maples and buckeyes that the major part of the damage occurred on the northwest and north sides of the trees,

partly because the wind was from that direction, and partly, as will be shown later, from another cause. It was observed that the line of demarcation between the frozen and unfrozen portions of the leaf always followed the outline of the latter, so that, minus the frozen portion, the remainder had all the appearances of a true leaf. In the magnolias of this class the frozen margin has acted as a limiting band and the further growth of the leaf has caused an arching of the surface towards the upper side in *Magnolia Lennei* ×, and towards the lower side in *Magnolia acuminata.* The latter, while included in the next section, is perhaps as truly a member of this. It is somewhat later than other members of the Magnoliaceae and had but few leaves exposed at the time of the frost. These were killed. The remainder, whose edges were more or less exposed in the opening bud, showed the effects mentioned above. That there exists a difference in the response of *Acer* and *Magnolia* to the same stimulus must be due to the difference in the maturity of the leaves, the former being probably more mature than the latter, and, consequently, incapable of growth recovery later, while in the latter, growth proceeded even after the leaf had been injured. Some new bud growth was induced, but since the effect was on trees that had already produced their spring crop of leaves, we may conclude that, in the absence of extensive new production of foliage, the productive ability of the remaining leaf surface would be much reduced, and possibly even to such an extent, that its effect would be perceptible in a difference in the size of the annual rings of the trees, or in a reduced activity in the foliar production of the following year, *i. e.,* in earliness and abundance of flowers and leaves, etc.

SECTION III.

We are forced to include this section, though the effect of the frost differs but in degree from the former division, mainly for the reason that we have here a method of

recovery that does not occur in the former case, nor in the one following. We are here concerned only with the origin of new growths, since all exposed growths have been completely killed.

Liriodendron tulipifera. All unfolded leaves were, of course, completely killed. Each leaf is, however, unfolded independently from the former one, and separated from it by the protective opening scales. Those so protected by the scales proceeded to grow, and, although a long time developing completely, were among the first to start, since their growing period was not shortened. The dropping off of foliage later in the season cannot be taken as any frost indication, since this condition occurs nearly every season, probably as a result of smoke injury.

Ginkgo biloba. This is a broad-leaved Gymnosperm which bears its leaves in clusters, of which the outer leaves are the older, and the inner the younger. The new leaves come forth from the center of the cluster one at a time. The freeze killed all of the opened leaves, which then turned brown and hung on until the middle of summer. The new leaves started to come out again within a few days after the frost, but their appearance was largely hidden until after the falling of the killed leaves. At the end of the season their appearance was much better than those of *Liriodendron.*

In both of the above cases the recovery has been rapid solely because the growth of new leaves did not involve the production of new buds, but simply a continuation of development in the buds that had already started, or more correctly, in the development of the uninjured shoot.

Upon the death of many of the leaves on shoots which were not injured, there was an immediate development of the unopened buds, as was the case with *Cercis canadensis* and *Cladrastis tinctoria.* In the latter the leaflets shrivelled up and fell off, the petiole remaining for some weeks, when it, too, fell, or was pushed off by the bud enclosed in its base. In this case we have a development of four separate

series of buds. First, those giving rise to the new shoot; second, those giving rise to the compound leaf; third, those designed for new growth the following year and enclosed within the bases of the leaves; and fourth, the so-called adventitious buds. The same condition also obtains for *Cercis canadensis*. In *Cladrastis* a drying up of the new shoot was noticed near the tip later in the season, but it cannot be said whether this was the result of frost injury, or of the drying effect of an unusually warm summer.

SECTION IV.

This is a somewhat arbitrary division, but by the death of the major portion of the shoot the appearance upon recovery was not as natural as that of the former section, inasmuch as the new leaves had a more clustered appearance. As a matter of fact the freeze cannot be said to have been injurious, any more than we might say the same for a good pruning. By freezing back the ends of the new shoots and entirely killing all new leaf growth, the frost forced new growth from the basal portion of the new shoot, and very largely from the adventitious buds on the old woody portion of the trees and shrubs. The appearance of *Ailanthus glandulosa, Aralia spinosa, Fraxinus, Rhus glabra* and *Rhus typhina,* have never been better than during the season following the freeze. Recovery has been rapid in nearly all cases, and apparently the freeze caused very little harm to this group. *Aralia spinosa,* in a clump on low ground, did not flower at all during the season, while another clump on higher ground flowered profusely. Here again, we cannot definitely place the cause of the difference, for normal growth causes might have resulted in an off year for flowering, as is the case with many of the Rosaceae.

SECTION V.

The last list is perhaps the most interesting of all, since it shows the effect of the freeze upon the new growth of

the Coniferae. The new growths at this time were just started, and they were entirely killed. Of course the old growth of leaves, even if frozen, did not show any effects. In *Broussonetia papyrifera, Catalpa bignonioides, Paulownia imperialis,* the killing was perhaps the most complete of all the trees and shrubs observed, in the case of the latter even extending into the old wood itself. *Magnolia grandiflora* is not hardy in this section, and only thrives when protected in winter. This protection had, of course, been removed and the new growths, as might have been expected, caught the full force of the freeze. It, however, responded well, and growth during the season was nearly normal. *Paulownia,* in the season from April 24 to the time of the first fall frost, October 28, made growths of ten to twelve feet. It, too, is but half hardy and freezes back a little nearly every winter and, strangely, after having passed the winter without freezing back, it did so, in some cases, as a result of the spring freeze, as evidenced by the appearance of the buds on the old wood. This is probably owing to the fact that growing tissue is much more susceptible to cold than is older tissue.

In recording the effects of the freeze on the many trees and shrubs of the list, several reasons have suggested themselves that partially explain why some leaves froze and why some did not. *(a)* Perhaps the most suggestive answer is the one of sap density. It is a well-known law of physics that solutions of different density have different freezing points. The freezing point for water being 32°F., any aqueous solutions, such as plant juices or saps, will have freezing points below that of pure water. We may suppose, then, that differences of sap density may determine the relative resistance to frost or freezing. The answer to this suggestion will occupy the second part of this paper and will not be taken up further here. *(b)* The opening of the buds, and the development of shoots and leaves, extends through a period of several weeks. It is rather interesting to note that those plants unaffected by the frost

are among the first to open and develop their leaves in the spring, as are those only partly affected; and those which have been the hardest hit of all the trees and shrubs, were among the last of all to open and expand their leaves. If it were possible to tabulate the ages of leaves and the order of their opening, we might also get an answer to our question, in that the older the leaf is, the more resistant it becomes. The time consumed in the experiments on freezing point lowering left no opportunity for complete work on this subject, and, except for the general confirmation of this supposition, nothing further has been done on the subject. *(c)* If, then, the ages of the leaves in general become a criterion of their resistance to freezing, then the difference in the ages of the parts of the leaves may account for the partial freezing of maples, etc., noticed in Section II. *(d)* Since the minimum of temperature recorded occurred during the night, we may also assume another cause. In the transformation of starch into sugar, which goes on in the leaf at night (as well as in the daytime), the plant is acquiring for itself a medium with a lower freezing point, for the insoluble starch grains have been changed into sugar, which, in solution, is gradually removed from the leaf. Such being the case the sugar solution would leave the tips of the leaves first and the more concentrated part would be near the center of the leaf. In other words the freezing point is lower at night, or at any time that respiration is greater than photosynthesis, in the center of the leaf than at the edge. It is probable that we have here a possible explanation of the partial freezing noticed in the leaves of *Acer* and *Aesculus*.

2. THE FREEZING POINTS OF LEAF SAPS, DETERMINED ARTIFICIALLY, AND NOTES IN CONNECTION THEREWITH.

At the time when the writer was expecting to make use of the rather cumbersome method of Beckman for determining the freezing point lowering of the cell saps, a paper appeared by Messrs. Dixon and Atkins, (Osmotic Pressure in Plants. Notes from the Botanical School of Trinity Col-

lege, Dublin **2**: 47-83. 1910.) in which they describe the preparation and use of the apparatus used in these experiments. A photograph of the complete apparatus used appears on the accompanying plate. It consists, in the main, of a thermo-couple made up of nickel wire, the ends of which are soldered to copper leads which connect with the terminals of the galvanometer. The junctions are fitted into two small glass tubes into one of which the solution to be frozen is placed (2-3cc.), and in the other, distilled water. The tubes are mounted in such a way that they can be inserted into a convenient vessel, which may be surrounded by the freezing mixture. The couple itself is supported by wooden rods so as to allow of constant stirring during the operation, and for removal and cleaning of both the junctions and the tubes. A reversing key, consisting of an ordinary spring clip, containing the leads from the galvanometer on its inner jaws, and the leads from the couple on the surface of a glass plate, is inserted in the circuit, and the whole submerged in coal oil to eliminate the effect of possible differences of temperature of the terminals of the galvanometer. A diagram of the apparatus is shown in the paper already referred to. The only essential difference in the apparatus as used, occurs in the use of German silver wire in the place of nickel in the thermo-couple, and in the use of a Leeds and Northrup Co. (Philadelphia) galvanometer, instead of the Ayrton-Mather instrument used by Dixon and Atkins. In using the apparatus it is essential that the whole of the couple be at the temperature of ice, for if any part of it remains out of the freezing temperature it acts itself as a couple and results in an inaccurate reading. In practice, after the distilled water has been placed in one of the tubes and the sap to be tested in the other, the holder containing the tubes and the couple is placed in a freezing mixture of salt-water and ice. The mixture surrounding the potato tube of the illustration may be used for this purpose. In a few moments the ice crystals may be seen

forming in the tube containing the distilled water, and shortly after in the tube containing the sap. During this time the liquid in the tubes has been kept stirred by means of the rods holding the thermo-couple. The insulation on the wires of the couple should be examined frequently during the operation so as to remedy any defects caused by the rubbing of the wires on the sides of the tubes in stirring. As soon as crystallization has set in, the holder containing the couple and tubes is put into the potato tube, which all this time has been surrounded by the freezing mixture. The two terminals of the reversing key are connected and the latter inserted in the vessel of coal oil which is kept stirred. When the movements of the mirror of the galvanometer have ceased, the current is reversed, and the reading taken from the other side of the zero. It is well to again reverse and verify the first reading, since if either of the liquids in the tubes should be unfrozen the deflection will increase, or when the liquids are frozen solid a sudden falling off toward the zero will be noticed. The movement of the mirror after the leads have been connected should be steady and then come to a complete stop. Any deviation from this action in the way of erratic movements may be taken as an indication of poor insulation, or over- or under-freezing. A little practice will easily make the operator acquainted with the instrument, and once familiar with it, there will be no difficulty at all in obtaining accurate readings. The deflections of the galvanometer used were not very great, ranging from two to nine millimeters. The tenths of a millimeter were estimated, and, consequently, many of the freezing points given in the tables as identical, may, as a matter of fact, be slightly different. A more sensitive galvanometer is to be desired for accurate and definite results, but for the purpose in view where only the comparison is of importance, the instrument used is quite sufficiently accurate. It has been found that on the jar containing the freezing mixture, ice or frost will usually form. When the

mixture warms the frost first leaves the bottom of the jar, moving in a band toward the top. At the appearance of this symptom it is usually found advisable to refill the jar, as procedure, after this point is reached, is usually slow and uncertain. Again, after connecting the terminals of the reversing key, care should be taken not to handle the stirrer until all traces of oil have been removed from the hands, and splashing of the freezing mixture on the couple should be avoided for very obvious reasons. Perfect cleanliness and care should be exercised throughout the test.

While all of the above is more or less a repetition of the directions for using the apparatus as given by Messrs. Dixon and Atkins, still it seems necessary to repeat such essentials as have been given here.

Altogether, some 110 species of plants (trees and shrubs) have been gathered, and of these only 90 have been finally tested out, owing to the inability of getting sap from some, and to its viscidity in others, thereby preventing its use in the small tubes containing the couple. The plants whose saps, for one reason or another, could not be used in the tests are as follows:—

Crataegus prunifolius	Paulownia imperialis
Pyrus japonica	Sassafras officinalis
Acer platanoides	Ulmus campestris
Aesculus Hippocastanum	Acer laetum rubrum
Quercus imbricaria	Amorpha fruticosa
Pyrus arbutifolius	Euonymus atropurpureus
Acer saccharinum laciniatum	Hydrangea radiata
Ulmus alata	Ampelopsis quinquefolia
Leitneria floridana	Spiraea prunifolia
Tilia americana	

Owing to the slow process of sap extraction and the limited time at the disposal of the writer, it was found necessary to carry the experiments along in sections, *i. e.,* the plant leaves were collected from 20 to 30 species at a time, the saps pressed out by means of a vise, after first being bruised in a mortar and wrapped in muslin which had been shrunk several times. Steel plates, which are shown at the left

of the illustration of the instrument used, are a convenient means of conducting the sap from the jaws of the vise to the test tubes. The saps were placed in test tubes, stoppered with cotton, numbered, and then stored in an ice box for approximately twenty-four hours, after which they were frozen in exactly the same order in which they were collected. Since, according to Dixon and Atkins, the saps stored for a period of twenty-four hours have the same freezing points as when freshly extracted, it is assumed that this represents their normal freezing point. But even if this were not so, and the writer has not tried to verify their conclusion, then the results will all be in error in the same direction, and hence are, for our purpose, quite accurate enough. The saps were collected for a period of about a week, fortunately accompanied by rather uniform weather, mostly clear, although cloudy at times, and not accompanied by rains at any time during the experiments. However, the weather was exceedingly dry, and it was at times difficult to obtain perfectly turgid leaves. All of the leaves from which sap was taken, were gathered in full sunlight, and, since they were mostly taken between 2:00 and 4:00 P. M., were from the southwest to the west side of the trees and shrubs. Except for some of the lower growing shrubs, the leaves were gathered at a point from four to five feet from the ground. The variation in color observed by Dixon and Atkins was also clearly shown by the saps after they had been allowed to settle for some time. The color of the sap when first pressed out and that observed after settling, *i. e.,* after the clear liquid had separated out from the chlorophyll and sediment, was quite variable, but since it had, as far as could be determined, exerted no influence on the freezing point lowering or had any relation to the results obtained, the tabulation of these colors has not been observed in this paper.

The times of collection, together with the dates of freezing, are as follows:—

Numbers 1 to 30. Gathered and sap extracted from 11 to 4 P. M., May 13, 1911: saps frozen 1 to 4 P. M., May 15, 1911.

Numbers 31 to 50. Collected May 17, 1911, from 2 to 4 P. M.: frozen 8 to 11 P. M., May 18, 1911.

Numbers 51 to 70. Collected May 18, 1911, from 2 to 4 P. M.: frozen 7 to 11 P. M., May 19, 1911.

Numbers 71 to 92. Collected May 19, 1911, from 2 to 4 P. M.: frozen 9 to 12 A. M., May 21, 1911.

Numbers 93 to 110. Collected May 20, 1911, from 2 to 4 P. M.: frozen 2 to 4 P. M., May 23, 1911.

The saps, with their freezing point lowering in degrees centigrade, are given in the order of their collection. Numbers from 1 to 10 are with thermo-couple number 1, in which one millimeter of deflection equals 0.2361°C., and numbers 11 to 110 are with couple number 2, in which one millimeter of deflection equals 0.29°C., calibrated with the sugar solutions of 10, 20, 30, and 40 grams to 100cc. of water, taking Raoult's lowering for each as respectively 0.58°, 1.16°, 1.74°, and 2.32° centigrade.

		Degrees Centigrade
1.	Cornus Mas	1.771
2.	Hydrangea paniculata grandiflora	1.322
3.	Magnolia Yulan	0.7792
4.	Magnolia cordata	1.2985
5.	Magnolia stellata	1.2513
6.	Magnolia glauca	0.8972
7.	Magnolia acuminata	0.8736
8.	Magnolia Kobus	1.1569
9.	Magnolia Soulangeana	1.2513
10.	Magnolia obovata (M. purpurea)	0.9444
11.	Morus alba	0.87
12.	Vitis vinifera	0.625
13.	Berberis vulgaris Thunbergii	1.45
14.	Crataegus prunifolius (no sap).	
15.	Aralia spinosa	1.363
16.	Ribes aureum	1.16
17.	Hibiscus syriacus	1.102
18.	Viburnum Lentago	1.943

		Degrees Centigrade
19.	Hydrangea quercifolia	0.9325
20.	Cercis canadensis	0.87
21.	Viburnum Opulis sterilis	1.74
22.	Philadelphus gordonianus	1.208
23.	Euonymus Bungeanus	1.305
24.	Pyrus Malus	1.885
25.	Dirca palustris	0.957
26.	Symphoricarpos racemosus	1.624
27.	Euonymus europaeus	1.856
28.	Lindera Benzoin	1.16
29.	Zelkova crenata	1.015
30.	Zanthoceras sorbifolia	1.16
31.	Cornus stolonifera	1.276
32.	Liquidambar styraciflua	1.131
33.	Pyrus japonica (no sap).	
34.	Aesculus austrina (sunlight)	1.073
35.	Aesculus austrina (shade)	0.918
36.	Viburnum dentatum	1.421
37.	Forsythia suspensa	1.073
38.	Halesia tetraptera	1.208
39.	Spiraea bracteata	2.03
40.	Cladrastis tinctoria	1.498
41.	Catalpa bignonioides	1.044
42.	Diervilla rosea var.	0.87
43.	Maclura aurantiaca	1.247
44.	Syringa vulgaris var.	1.74
45.	Acer platanoides (no sap).	
46.	Fagus sylvatica purpurea	1.208
47.	Syringa persica	1.914
48.	Aesculus parviflora	1.131
49.	Aesculus Hippocastanum (no sap).	
50.	Viburnum tomentosum	0.957
51.	Magnolia obovata discolor	1.208
52.	Ginkgo biloba	1.45
53.	Chionanthus virginicus	1.015
54.	Hydrangea paniculata	1.305
55.	Rhus cotinus atropurpureus	1.208
56.	Viburnum Opulis sterilis	1.624
57.	Platanus occidentalis	1.302
58.	Quercus imbricaria (no sap).	
59.	Pyrus arbutifolia (no sap).	
60.	Magnolia Lennei ×	0.741
61.	Euonymus Sieboldianus	1.16
62.	Acer dasycarpum (no sap).	

		Degrees Centigrade
63.	Rhamnus tinctoria	1.74
64.	Ilex opaca	0.596
65.	Hamamelis virginica	1.16
66.	Clethra alnifolia	1.073
67.	Philadelphus coronaria	1.131
68.	Philadelphus grandifolius	1.16
69.	Koelreuteria paniculata	1.131
70.	Sambucus canadensis	1.566
71.	Liriodendron tulipifera	1.305
72.	Acer saccharinum laciniatum (no sap).	
73.	Ulmus alata (no sap).	
74.	Carpinus Betula	1.479
75.	Magnolia Umbrella	0.87
76.	Leitneria floridana (no sap).	
77.	Ostrya virginica	1.45
78.	Diospyros virginiana	1.015
79.	Taxodium distichum	1.073
80.	Asimina triloba	0.625
81.	Tilia americana (no sap).	
82.	Fraxinus Ornus	1.363
83.	Ligustrum amurense	1.74
84.	Broussonetia papyrifera	1.16
85.	Fraxinus americana	0.712
86.	Ailanthus glandulosus	1.16
87.	Paulownia imperialis (no sap).	
88.	Rhus typhina	1.015
89.	Rhus glabra	1.276
90.	Sassafras officinalis (no sap).	
91.	Ulmus campestris (no sap).	
92.	Acer laetum rubrum (no sap).	
93.	Gymnocladus canadensis	1.74
94.	Fraxinus viridis	1.208
95.	Cornus Baileyi	1.276
96.	Neillia opulifolia	1.015
97.	Gleditschia aquatica	1.305
98.	Amorpha fruticosa (no sap).	
99.	Euonymus atropurpurea (no sap).	
100.	Berberis ilicifolia	1.566
101.	Calycanthus floridus	1.392
102.	Rhus copallina	1.305
103.	Juglans rupestris	1.015
104.	Magnolia grandiflora	1.044
105.	Hydrangea radiata (no sap).	
106.	Securinega fluggeoides	1.566

		Degrees Centigrade
107.	Ampelopsis quinquefolia (no sap).	
108.	Spiraea prunifolius (no sap).	
109.	Gleditschia triacanthos	1.595
110.	Spiraea Van Houttei	1.566

The following table is the arrangement of the species in the order of their freezing point lowering. The numbers following the names of the species represent the respective section to which they were referred in the tentative lists arranged directly after the freeze of 1910:—

		Degrees Centigrade
1.	Spiraea bracteata	2.03
2.	Viburnum Lentago (2)	1.943
3.	Syringa persica (1)	1.014
4.	Pyrus Malus	1.885
5.	Euonymus europaeus	1.856
6.	Cornus Mas (1)	1.7708
7.	Gymnocladus canadensis (4)	1.74
8.	Ligustrum amurense (2)	
9.	Rhamnus tinctoria (4)	
10.	Syringa vulgaris var. (1)	
11.	Viburnum Opulis sterilis (2)	
12.	Viburnum Opulis sterilis (2)	1.624
13.	Symphoricarpos racemosus	
14.	Gleditschia triacanthos (4)	1.595
15.	Spiraea Van Houttei (4)	1.566
16.	Securinega fluggeoides (4)	
17.	Berberis ilicifolia (4)	
18.	Sambucus canadensis (3)	
19.	Cladrastis tinctoria (3)	1.498
20.	Carpinus Betulus (4)	1.479
21.	Ostrya virginica (2)	1.45
22.	Ginkgo biloba (3)	
23.	Berberis vulgaris Thunbergii (4)	
24.	Viburnum dentatum	1.421
25.	Calycanthus floridus (3)	1.392
26.	Platanus occidentalis (2)	
27.	Fraxinus Ornus (4)	1.363
28.	Aralia spinosa (4)	
29.	Hydrangea paniculata grandiflora (2)	1.3222
30.	Rhus copallina (1)	1.305
31.	Gleditschia aquatica (4)	
32.	Liriodendron tulipifera (3)	

		Degrees Centigrade
33.	Hydrangea paniculata (2)	
34.	Euonymus Bungeanus	
35.	Magnolia cordata (2)	1.2985
36.	Cornus Baileyi (2)	1.276
37.	Rhus glabra (4)	
38.	Cornus stolonifera (1)	
39.	Magnolia Soulangeana (2)	1.2513
40.	Magnolia stellata (1)	
41.	Maclura aurantiaca (3)	1.247
42.	Fraxinus viridis (4)	1.208
43.	Rhus cotinus atropurpureus (5)	
44.	Magnolia obovata discolor (2)	
45.	Fagus sylvatica purpurea (2)	
46.	Halesia tetraptera (2)	
47.	Philadelphus gordonianus	
48.	Ailanthus glandulosus (4)	1.16
49.	Broussonetia papyrifera (5)	
50.	Philadelphus grandiflorus (2)	
51.	Hamamelis virginica (2)	
52.	Euonymus Sieboldianus (1)	
53.	Zanthoceras sorbifolia (3)	
54.	Lindera Benzoin (4)	
55.	Ribes aureum (2)	
56.	Magnolia Kobus (1)	1.1569
57.	Koelreuteria paniculata (3)	1.131
58.	Philadelphus coronarius (2)	
59.	Aesculus parviflora (2)	
60.	Liquidambar styraciflua (4)	
61.	Hibiscus syriacus (5)	1.102
62.	Taxodium distichum (2)	1.073
63.	Clethra alnifolia (1)	
64.	Forsythia suspensa	
65.	Aesculus austrina (sunlight)	
66.	Magnolia grandiflora (5)	1.044
67.	Catalpa bignonioides (5)	
68.	Juglans rupestris (4)	1.015
69.	Neillia opulifolia (2)	
70.	Rhus typhina (4)	
71.	Diospyros virginiana (5)	
72.	Chionanthus virginicus (5)	
73.	Zelkova crenata	
74.	Viburnum tomentosum	0.957
75.	Dirca palustris	
76.	Magnolia obovata (M. purpurea) (2)	0.9444

		Degrees Centigrade
77.	Hydrangea quercifolia (3)	0.9325
78.	Aesculus austrina (shade)	0.918
79.	Magnolia glauca (2)	0.8972
80.	Magnolia acuminata (3)	0.8736
81.	Magnolia Umbrella (2)	0.87
82.	Diervilla rosea var. (2)	
83.	Cercis canadensis (3)	
84.	Morus alba (4)	
85.	Magnolia Yulan (3)	0.7792
86.	Magnolia Lennei × (2)	0.741
87.	Fraxinus americanus (4)	0.712
88.	Asimina triloba (5)	0.625
89.	Vitis vinifera (5)	
90.	Ilex opaca (5)	0.596

By dividing the above list, in which the species are arranged in descending order, according to the respective freezing point lowerings, into halves, and by listing under each half the number of species therein belonging to the sections outlined in the early part of this paper, the following arrangement results:—

First Half.	Second Half.
6 of Section I.	3 of Section I.
13 of Section II.	13 of Section II.
6 of Section III.	6 of Section III.
13 of Section IV.	7 of Section IV.
0 of Section V.	10 of Section V.

In the above the absence of any of Section V from the first half is very noticeable, and only a small fraction of a degree keeps the three of Section I in the second half. Those belonging to Section III, as might be expected, are evenly distributed in both halves, since they represent the intermediate position between Sections I and V. However, the large number of Section II in the second half, and the large number of Section IV in the first half seems to be rather inconsistent, although when we take into consideration the fact that these sections represent purely arbitrary divisions, it is not to be given much thought.

The greatest freezing point lowering, 2.03°C., and the least freezing point lowering, 0.596°C., constitute but a small range in temperature. Nevertheless, within this range there occur several similarities of conduct under varying conditions, to which it may be well to call attention here. *Viburnum Opulus sterilis,* collected and frozen on separate days from different plants, vary but 0.116°C. from each other. However, in *Aesculus austrina,* collected in the sun, and that collected in the shade at the same time and from the same tree, there is a difference of 0.155°C., the lowest freezing point lowering being for the sample from sunlight. The maximum lowering of 1.2985°C., and the minimum of 0.741°C., for *Magnolia cordata* and *Magnolia Lennei* × respectively, indicates a wide difference in the behavior of this group. Their relations from the foregoing table are here submitted for more graphic comparison:—

1910 Observations.	1911 Freezing Tests.
M. stellata	M. cordata
M. Kobus	M. stellata
M. cordata	M. Soulangeana
M. Soulangeana	M. obovata discolor
M. obovata discolor	M. Kobus
M. obovata purpurea	M. grandiflora
M. glauca	M. obovata purpurea
M. Umbrella	M. glauca
M. Lennei ×	M. acuminata
M. acuminata	M. Umbrella
M. Yulan	M. Yulan
M. grandiflora	M. Lennei ×

Taking the observations of 1911 as a basis, there would seem to be a very close relation between the behavior of this group in actual and artificial conditions. With the exception of *Magnolia grandiflora* and, perhaps, of *Magnolia Kobus,* the lines connecting the members of the two groups diverge but little. The impression seems to be given that within species of the same genus, differences in the density of the cell sap result in differences in the resistance of the plants to freezing weather, and the relative relation of the species in this respect may be determined by a knowledge of the freezing points of their cell saps, obtained under

the same conditions of light, exposure, etc. It is quite probable that the differences noted above may have been caused by the failure, in either of the two seasons, to take account of the relative protective positions of the trees from which specimens for sap determination were taken.

3. CONCLUSIONS.

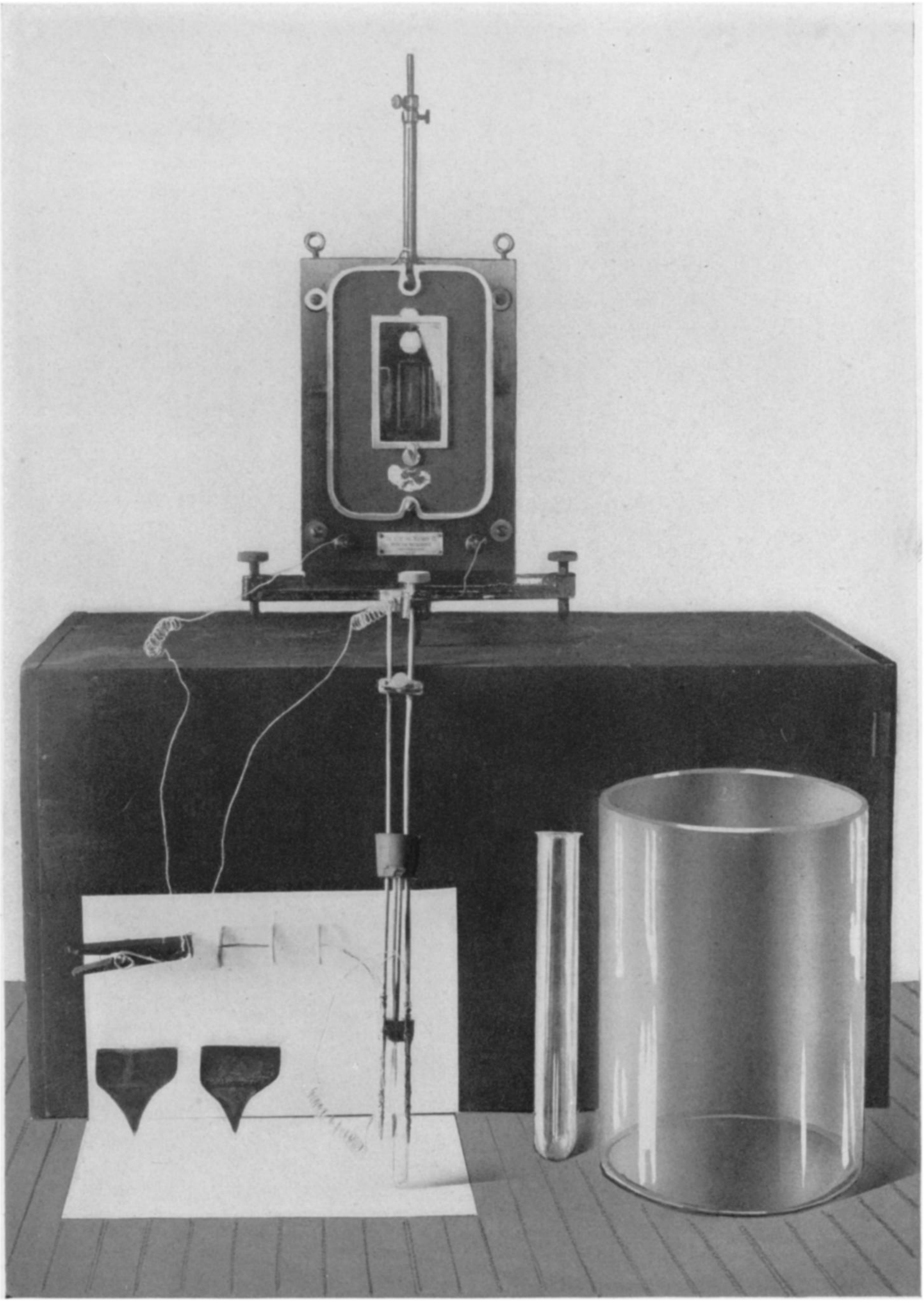
Our conclusions are based upon two series of investigations: one, the observations of the effect of a freeze on many trees and shrubs; the other, the artificial freezing of the leaf saps of the above trees and shrubs. The first is more or less arbitrary, being based mostly on external appearances; the second is hypothetically accurate. From a comparison of these two sets of data we are led to the following conclusions:—

1. That extreme differences in sap density, in general, are accompanied by a corresponding difference in their resistance to freezing.

2. That exceptions to this general rule are probably due to differences of cell structure, and other causes that may enter in, as protective location, etc.

3. That where cell structure is the same, the densities of the cell saps indicate their relative hardiness, as in the magnolias.

4. That in plants of the same genus, or varieties of the same species, differences in sap density correspond to differences in their resistance to freezing.



FREEZING APPARATUS.

THE ARM OF THE GALVANOMETER HAS BEEN REMOVED.